

CLAIMS

1 1. A process for inhibiting misincorporation of a terminator in a
2 single base primer extension reaction, comprising the steps of:
3 providing a product of a nucleic acid synthesis reaction, the product
4 comprising a nucleic acid template and a quantity of inorganic pyrophosphate;
5 incubating the product and an inorganic pyrophosphatase under
6 conditions sufficient to decrease the quantity of pyrophosphate, to yield a
7 purified reaction product;
8 combining the purified reaction product, a primer, a terminator having a
9 detectable label, and a polymerase to form a mixture; and
10 incubating the mixture under conditions sufficient to extend the primer
11 by addition of the terminator in a single base primer extension reaction,
12 wherein decreasing the quantity of inorganic pyrophosphate in the product of a
13 nucleic acid synthesis reaction inhibits pyrophosphorolysis in the single base
14 primer extension reaction, so as to inhibit misincorporation of a terminator.

1 2. The process of claim 1 wherein the nucleic acid synthesis
2 product further comprises a residual reaction component selected from the
3 group consisting of: a residual primer and a nucleotide.

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1 3. The process of claim 2 further comprising the steps of:
2 adding an enzyme selected from the group consisting of: an
3 exonuclease, an alkaline phosphatase, and a combination thereof to the nucleic
4 acid synthesis product; and
5 incubating the nucleic acid synthesis product and enzyme under
6 conditions sufficient to degrade the residual reaction component.

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1 4. The process of claim 2 further comprising the steps of:
2 adding an enzyme selected from the group consisting of: an
3 exonuclease, an alkaline phosphatase, and a combination thereof to the purified
4 reaction product; and

5 incubating the nucleic acid synthesis product and enzyme under
6 conditions sufficient to degrade the residual reaction component.

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1 5. The process of claim 3 or 4 further comprising the step of:
2 inactivating the enzyme.

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1 6. The process of claim 1 further comprising the step of
2 inactivating the inorganic pyrophosphatase.

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1 7. The process of claim 1 wherein the detectable label is a
2 fluorescent label.

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1 8. The process of claim 1 wherein the detectable label is selected
2 from the group consisting of: an isotopic moiety, a mass tag, a peptide moiety,
3 a carbohydrate moiety and a combination thereof.

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1 9. The process of claim 1 further comprising the step of detecting
2 the detectable label.

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1 10. The process of 9 wherein the step of detecting the label
2 comprises detection of fluorescence polarization.

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1 11. The process of claim 9 wherein the step of detecting the label
2 comprises direct fluorescence detection, fluorescence quenching, fluorescence
3 anisotropy, time resolved fluorescence and fluorescence energy transfer.

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1 12. The process of claim 9 wherein the step of detecting the label
2 comprises detection selected from the group consisting of: radiation detection,
3 mass spectrometry, and chromophore detection.

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1 13. The process of claim 3 or 4 wherein the alkaline phosphatase is
2 selected from the group consisting of: bacterial alkaline phosphatase, calf
3 intestinal alkaline phosphatase and a combination thereof.

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1 14. The process of claim 3 or 4 wherein the alkaline phosphatase is
2 shrimp alkaline phosphatase.

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1 15. The process of claim 3 or 4 wherein the exonuclease is selected
2 from the group consisting of: lambda exonuclease, mung bean exonuclease,
3 Bal31 exonuclease, T7 exonuclease and a combination thereof.

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1 16. The process of claim 3 or 4 wherein the exonuclease is
2 exonuclease I.

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1 17. The process of claim 3 or 4 wherein the enzyme is a
2 combination of shrimp alkaline phosphatase and exonuclease I.

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1 18. The process of claim 1 wherein the polymerase is a
2 thermostable polymerase having a greater affinity for an acyclo nucleoside
3 terminator than for a dideoxyterminator.

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1 19. The process of claim 1 wherein the inorganic pyrophosphatase
2 is selected from the group consisting of: a mammalian inorganic
3 pyrophosphatase, a bacterial inorganic pyrophosphatase, a yeast inorganic
4 pyrophosphatase, and a combination thereof.

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1 20. The process of claim 1 wherein the inorganic pyrophosphatase
2 is a thermostable inorganic pyrophosphatase.

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1 21. The process of claim 1 wherein the steps are performed in a
2 single reaction container.

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1 22. The process of claim 1 wherein the primer is included in a
2 primer array.

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1 23. The process of claim 1 wherein the terminator is an acyclo
2 nucleoside terminator.

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1 24. The process of claim 1 wherein the acyclo nucleoside terminator
2 comprises a detectable label.

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1 25. The process of claim 1 wherein the detectable label is a
2 fluorescent label.

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1 26. A process for inhibiting misincorporation of a terminator in a
2 single base primer extension reaction, comprising the steps of:

3 providing a product of a nucleic acid synthesis reaction, the product
4 comprising a nucleic acid template and a quantity of inorganic pyrophosphate;

5 incubating the product and a pyrophosphate removing enzyme under
6 conditions sufficient to decrease the quantity of pyrophosphate, to yield a
7 purified reaction product;

8 combining the purified reaction product, a primer, a terminator having a
9 detectable label, and a polymerase to form a mixture; and

10 incubating the mixture under conditions sufficient to extend the primer
11 by addition of the terminator in a single base primer extension reaction,
12 wherein decreasing the quantity of inorganic pyrophosphate in the product of a
13 nucleic acid synthesis reaction inhibits pyrophosphorolysis in the single base
14 primer extension reaction, so as to inhibit misincorporation of a terminator.

1 27. The process of claim 26 wherein the nucleic acid synthesis
2 product further comprises a residual reaction component selected from the
3 group consisting of: a residual primer and a nucleotide.

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1 28. The process of claim 27 further comprising the steps of:
2 adding an enzyme selected from the group consisting of: an
3 exonuclease, an alkaline phosphatase, and a combination thereof to the nucleic
4 acid synthesis product; and
5 incubating the nucleic acid synthesis product and enzyme under
6 conditions sufficient to degrade the residual reaction component.

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1 29. The process of claim 27 further comprising the steps of:
2 adding an enzyme selected from the group consisting of: an
3 exonuclease, an alkaline phosphatase, and a combination thereof to the purified
4 reaction product; and
5 incubating the nucleic acid synthesis product and enzyme under
6 conditions sufficient to degrade the residual reaction component.

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1 30. The process of claim 26 further comprising the step of
2 inactivating the inorganic pyrophosphatase.

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1 31. The process of claim 26 wherein the pyrophosphate removing
2 enzyme is selected from the group consisting of: a pentosyltransferase, a
3 phosphotransferase, a nucleotidyl transferase and a carboxylase.

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1 32. A process for inhibiting misincorporation of a terminator in a
2 single base primer extension reaction, comprising the steps of:

3 combining a nucleic acid template, a primer, an inorganic
4 pyrophosphatase, an acyclo nucleoside terminator, and a polymerase to yield a
5 mixture substantially free of deoxynucleotide-triphosphates; and

6 incubating the mixture under conditions sufficient to extend the primer
7 by addition of the acyclo nucleoside terminator, wherein the pyrophosphatase
8 inhibits pyrophosphorolysis in the single base primer extension reaction,
9 thereby reducing misincorporation of a terminator.

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1 33. The process of claim 32 wherein the polymerase has higher
2 affinity for an acyclo nucleoside terminator than for a dideoxynucleotide
3 terminator.

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1 34. The process of claim 32 wherein the polymerase is a
2 thermostable polymerase.

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1 35. The process of claim 32 wherein the primer comprises a 3'
2 terminal nucleotide complementary to the interrogation site nucleotide.

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1 36. The process of claim 32 wherein the primer comprises a
2 nucleotide complementary to the interrogation site and wherein the nucleotide
3 is 2-10 nucleotides upstream of the 3' terminal nucleotide of the primer.

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1 37. The process of claim 32 wherein terminator is an acyclo
2 nucleoside terminator.

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1 38. The process of claim 32 wherein the acyclo nucleoside
2 terminator comprises a detectable label.

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1 39. The process of claim 38 wherein the detectable label is a
2 fluorescent label.

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1 40. A composition, comprising:
2 an inorganic pyrophosphatase;
3 a residual component removal agent selected from the group consisting
4 of: an alkaline phosphatase, an exonuclease, and a combination thereof; and
5 a carrier.

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1 41. The composition of claim 40 wherein the ratio of enzyme
2 activity units of residual component removal agent to enzyme activity units of
3 inorganic pyrophosphatase ranges between 1000:1 – 1:1000.

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1 42. The composition of claim 40 wherein the ratio of enzyme
2 activity units of residual component removal agent to enzyme activity units of
3 inorganic pyrophosphatase ranges between 100:1 – 1:100.

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1 43. The composition of claim 40 wherein the ratio of enzyme
2 activity units of residual component removal agent to enzyme activity units of
3 inorganic pyrophosphatase ranges between 10:1 – 1:10.

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1 44. The composition of claim 40 wherein the alkaline phosphatase
2 is selected from the group consisting of: bacterial alkaline phosphatase, calf
3 intestinal alkaline phosphatase and a combination thereof.

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1 45. The composition of claim 40 wherein the alkaline phosphatase
2 is shrimp alkaline phosphatase.

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1 46. The composition of claim 40 wherein the exonuclease is
2 selected from the group consisting of: lambda exonuclease, mung bean
3 exonuclease, Bal31 exonuclease, T7 exonuclease and a combination thereof.

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1 47. The composition of claim 40 wherein the exonuclease is
2 exonuclease I.

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1 48. A composition for use in reducing misincorporation of a
2 terminator in a single base extension reaction, comprising:

3 an acyclo nucleoside terminator;

4 an inorganic pyrophosphate;

5 a pyrophosphatase; and
6 a carrier.

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1 49. The composition of claim 48 wherein the acyclo nucleoside
2 terminator comprises a detectable label.

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1 50. The composition of claim 48 wherein the pyrophosphatase is a
2 yeast inorganic pyrophosphatase.

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1 51. The composition of claim 48 wherein the pyrophosphatase is
2 selected from the group consisting of: a bacterial inorganic pyrophosphatase
3 and a mammalian inorganic pyrophosphatase.

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1 52. A commercial package comprising:
2 a mixture of an exonuclease, an alkaline phosphatase, an inorganic
3 pyrophosphatase, and a carrier; and
4 instructions for use of the mixture in a primer extension reaction.

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1 53. The commercial package of claim 52 wherein the exonuclease is
2 exonuclease I.

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1 54. The commercial package of claim 52 wherein the alkaline
2 phosphatase is shrimp alkaline phosphatase.

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1 55. The commercial package of claim 52 wherein the
2 pyrophosphatase is a yeast pyrophosphatase.

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1 56. The commercial package of claim 52 wherein the
2 pyrophosphatase is a thermostable pyrophosphatase.

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1 57. The commercial package of claim 52 wherein the
2 pyrophosphatase is selected from the group consisting of: a bacterial
3 pyrophosphatase and a mammalian pyrophosphatase.

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1 58. The commercial package of claim 52 wherein the mixture
2 further comprises an additive selected from the group consisting of: a chelator,
3 a polyol, a reducing agent, a protease inhibitor, a detergent, and a combination
4 thereof.

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1 59. The commercial package of claim 52 wherein the carrier is a
2 buffered solution.

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1 60. Use of an inorganic pyrophosphatase in a process for
2 identification of an interrogation site by single base extension.

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1 61. A process for determining the identity of a nucleotide at an
2 interrogation site, essentially as described herein.

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1 62. A composition comprising an inorganic pyrophosphatase,
2 essentially as described herein.

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1 63. A commercial package comprising an inorganic
2 pyrophosphatase, essentially as described herein.